Dynamics and Genetics of Hippocampal Network Activity

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General Introduction

1.1 Introduction

“If the human brain were so simple that we could understand it, we would be so simple that we couldn't”. Emerson M. Pugh.

The human brain is by far the most complex structure that is found in the universe. The cerebral cortex alone contains 15–33 billion neurons (brain cells), each making roughly 10.000 synaptic connections with other neurons. A cubic millimeter of cerebral cortex contains around one billion synapses. Brain activity consists of chemical and electrical communication in this immense network that gives rise to our thoughts, memories, believes and dreams.

A prominent feature of the brain’s activity is the rhythmic synchronous firing of neurons, also called neuronal oscillations. These oscillations support many cognitive functions and are altered in several psychiatric disorders. Traits (properties) of these oscillations vary also a lot in healthy humans, and this variance is often for more than 50% caused by genetic factors. Cognitive ability and psychiatric diseases are also highly heritable, but it is difficult to find genes that influence these complex traits, because they are influenced by many genes. Oscillations are closer to the gene action, and therefore, genes that influence oscillations may be more easily identified. These genes may also shape higher level cognitive traits via the intermediate oscillations. The research in this thesis contributes to the field that aims at identifying the pathways from genes to oscillations to cognition. We analyzed neuronal oscillations in the hippocampus, a brain region involved in cognitive functions. We aimed to 1) thoroughly characterize hippocampal network activity with classical and non-classical quantitative traits 2) estimate the heritability of these traits 3) perform a genome wide search for genes that influence the hippocampal traits and 4) investigate with which behavioral or cognitive traits the hippocampal traits may be related.

1.2 Genes and Cognition

Cognition encompasses all processes of mental activity, such as thinking, memory, language and information processing, attention, perception and imagination. Humans vary widely in their cognitive abilities. This variability is caused by environmental and genetic factors. The percentage of the total variability that is explained by genetic factors is called the heritability. In a meta analysis of studies on the genetic influence on intelligence, that covered the first 50 years of the twentieth century, the correlation coefficients for intelligence between several types of relatives were collected (Erlenmeyer-Kimling & Jarvik, 1963), see Figure 1.1. It appeared that the closer the relative (in a genetic sense), the higher the

correlation. This was the first indication that genetic influences on intelligence are prominent. More recently, it was confirmed that cognitive abilities are highly heritable, ranging from about 40% for the performance on attention and working memory tasks (Cornblatt *et al.*, 1988; Hansell *et al.*, 2005), to about 80% for IQ in adults (Plomin & Spinath, 2004). Interestingly, different cognitive abilities are highly correlated, therefore it was argued that they can be represented by one general intelligence factor. It also led to the hypothesis of "generalist genes", which states that a single group of genes affects multiple aspects of cognition (Plomin & Kovas, 2005).

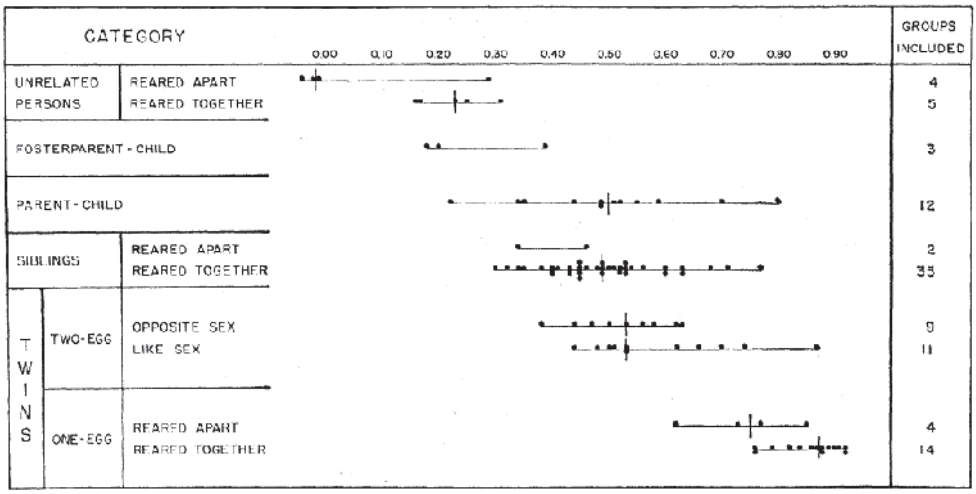

Figure 1.1. **Similarity in cognitive ability increases with similarity in genes.**
Black dots are correlation coefficients of cognitive ability from 52 studies. Cognitive ability is strongly correlated between monozygotic twins, and very weakly between unrelated persons, which indicates a strong genetic influence on cognitive ability (adapted from Erlenmeyer-Kimling *et al.*, 1963).

Since variability in cognitive ability is to a large extent due to genetic factors, many studies have been designed to find genes that influence cognition. Two main strategies can be followed for this purpose, genome wide linkage analysis and candidate-gene association studies. For both studies single nucleotide polymorphisms (SNPs) are measured. In genome wide linkage studies these genetic markers are measured over the entire genome in a group of individuals, in order to relate variation in the genomic markers with variation in a cognitive marker (Posthuma *et al.*, 2005). In candidate-gene association studies, only one or few candidate genes that are suspected to be involved are genotyped and correlated with cognitive markers (Comings *et al.*, 2003). Most of the studies identified several candidate genes, but the findings are difficult to replicate, and the identified genes explain maximally 1% of the phenotypic variance (Posthuma & De Geus, 2006; Deary *et al.*, 2009).

Thus although cognitive abilities are highly heritable, the gene finding strategies have had minor success so far, which has led to an ongoing discussion about the reasons for the "missing heritability" and of alternative strategies to identify these important genes (Eichler *et al.*, 2010). One reason for this limited success may be that cognitive traits are influenced by many genes, all with small effects, and therefore difficult to track down. Investigating groups of genes simultaneously and measuring the effect at group level instead of studying each of them individually, may increase the likelihood to detect genes (Ruano *et al.*, 2010). Another strategy aims at finding genes that influence physiological mechanisms that mediate the effect of genes on a complex trait, the so-called endophenotypes.

1.3 The endophenotype concept

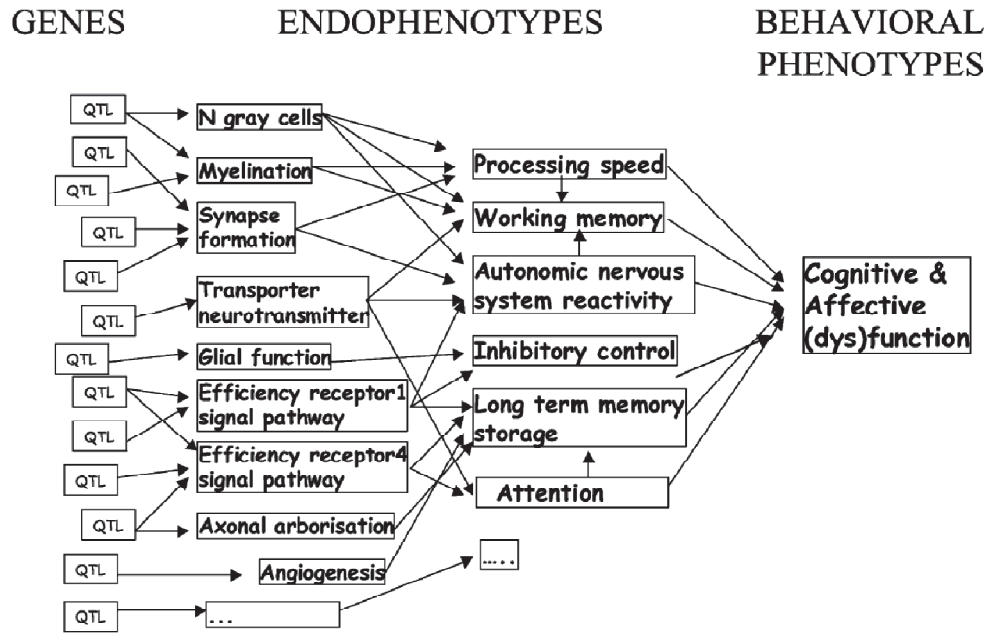

Figure 1.2. **Endophenotypes mediate the effect of genes onto complex traits**.
Each endophenotype mediates only part of all the genes involved, which thereby reduces the complexity of the gene finding, and provides insight into the pathways from gene to behavioral phenotypes (adapted from De Geus *et al.*, 2002).

The concept of the endophenotype was introduced by John & Lewis (1966) and later adapted by Gottesman & Shields (1973). According to the definition in (Gottesman & Gould, 2003), an endophenotype should be heritable, influenced by the same genes as a psychiatric disease, and also be present in healthy individuals. The idea is, that complex phenotypes are often influenced by many less complex mechanisms (endophenotypes), for which the genes that influence them may be easier to find (Fig. 1.2). The endophenotype concept has earned much popularity in

recent years: the number of citations for "endophenotype" is growing exponentially (Fig. 1.3). In psychiatry, many neurophysiological endophenotypes have been proposed for schizophrenia (cerebral abnormalities, EEG response, memory performance), alcoholism (EEG coherence), ADHD (attention deficits), and autism (spatial memory deficits), see (Flint & Munafo, 2007) for a review.

The endophenotype concept has turned out to be a useful one in many cases. For example, the P50 auditory evoked EEG response was successfully used as an endophenotype for schizophrenia. The P50 response is evoked by two brief auditory clicks separated by a half second. In healthy subjects, the EEG response to the second click is reduced compared to the response to the first click, but in schizophrenics this reduction is not present (Freedman *et al.*, 1987), which is associated with attentional impairment (Cullum *et al.*, 1993). In a genome wide search for genes that shape the P50 response, a DNA region that contains the gene CHRNA-7, which codes for the α7 nicotinic acetylcholine receptors, was identified (Freedman *et al.*, 1997). This gene was later also found to be involved in schizophrenia, in several candidate-gene association studies (Leonard *et al.*, 1998; De Luca *et al.*, 2004). Currently, α7 nicotinic agonists are tested for treatment of schizophrenia (Freedman *et al.*, 2008). Thus, by studying the genetics of the P50 response, a gene was discovered that also influences schizophrenia, which demonstrates the usefulness of the endophenotype concept.

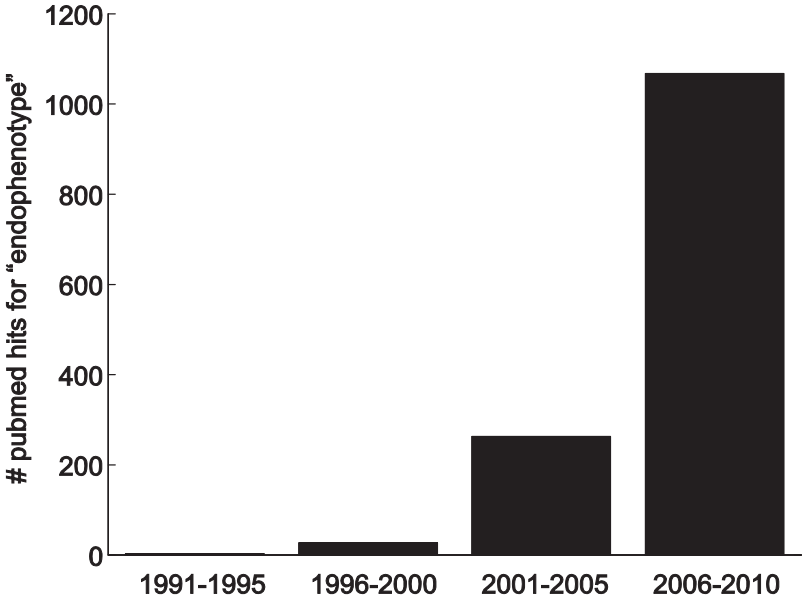

Figure 1.3. **The recent increase of the number of citations for the term endophenotype in PubMed.**

Although the endophenotype concept has gained much popularity, a meta-analysis of endophenotypes concluded that the effect sizes of genes contributing to endophenotypes are similar to those contributing to complex traits (Flint & Munafo, 2007). Thus, the genetic basis of endophenotypes and complex traits would be equally difficult to find. However, the endophenotype concept has wider applicability than just for finding genes for complex traits. It also serves to fill in gaps in the pathway from gene to trait, in order to understand the biology of the trait (Walters & Owen, 2007). If, for example, a gene is known to influence a complex trait, endophenotypes that mediate the genetic effect may be searched for. For example, COMT is a gene associated with schizophrenia (Williams *et al.*, 2007), for which the mechanism leading to the disease was largely unknown. The P300 EEG response appeared to be influenced by COMT, which shed some light on the possible pathway by which COMT contribute to schizophrenia. The P300 response is evoked by auditory or visual stimuli. Target stimuli (e.g., low tones) and non-target stimuli (e.g., high tones) are presented in a random order with 1 or 2 second interstimulus intervals. The subjects are asked to push a button if a target stimulus is detected. About 300 milliseconds after a target stimulus is detected, the EEG response increases. The amplitude and latency of this increase are the most commonly used parameters for quantifying the P300 response. In schizophrenics, the amplitude of the P300 response is smaller, and the latency larger compared to healthy subjects, which is associated with a reduced speed of cognitive processing (Roth & Cannon, 1972; Kang *et al.*, 2010). Candidate-gene association studies reported a link between COMT and the P300 response latency and amplitude (Gallinat *et al.*, 2003; Golimbet *et al.*, 2006; Yue *et al.*, 2009), which shows that the P300 can be used as an endophenotype that connects the gene COMT with schizophrenia, as a marker for cognitive processing speed.

Since the endophenotype concept originates from the field of psychiatry, in the original definition of Gottesman an endophenotype is always related to a psychiatric disease. Psychiatric diseases frequently come with cognitive impairment, therefore cognitive phenotypes (e.g. performance in cognitive tasks) have often been proposed as endophenotypes. But cognitive phenotypes are complex themselves, and De Geus and Boomsma (2001) argued that the endophenotype concept also applies to cognitive phenotypes. With our current understanding of brain function, almost any neurophysiological trait is a candidate endophenotype for cognitive phenotypes. Brain oscillations are believed to play an important role in cognition, can be measured with a wide range of techniques and in many brain regions, and thus are an important source of potential endophenotypes.

1.4 Neuronal oscillations: Endophenotypes for cognition and psychiatric disease?

One of the requirements for a trait to be an endophenotype is heritability. Neuronal oscillations have traditionally been categorized into five frequency bands: delta (1–

4 Hz), theta (4–8 Hz), alpha (8–13 Hz), beta (13–30 Hz) and gamma (30–100 Hz). Heritability of EEG amplitude in the classical frequency bands as recorded during a few minutes of eyes-closed rest ranges from 40 to 90% (Smit *et al.*, 2005). A significant heritability has also been reported for several of these oscillations in terms of their frequency (Posthuma *et al.*, 2001), coherence (Chorlian *et al.*, 2007; Smit *et al.*, 2010) and complex temporal structure of amplitude fluctuations (Linkenkaer-Hansen *et al.*, 2007). The auditory gamma response amplitude has a heritability of 65% (Hall *et al.*, 2009). Thus, neuronal oscillations contain many potential endophenotypes for the cognitive functions they are related with.

1.4.1 Cognitive functions of neuronal oscillations

Neuronal oscillations are present in various forms depending on the behavioral state. Delta band oscillations occur mostly during a sleep stage called slow-wave sleep (Steriade, 2003). Theta oscillations occur in response to stimuli, such as the P300 paradigm (Basar-Eroglu & Demiralp, 2001) and cooperate with gamma oscillations during memory formation (Lisman & Idiart, 1995; Axmacher *et al.*, 2010; Nyhus & Curran, 2010). Alpha rhythm is most prominent during rest, and a lot of effort has been made to find functional roles for them. In a review paper, (Palva & Palva, 2007) conclude that the amplitude of alpha oscillations cannot conclusively be related to a cognitive function, but that the phase relations of the alpha rhythm between brain regions might support cognition. Recent studies support this view, by showing that the phase synchrony of alpha oscillations between different brain regions is increasing with memory load (Haenschel *et al.*, 2010; Klimesch *et al.*, 2010; Palva *et al.*, 2010). Beta oscillations are related to the maintenance of the current cognitive or sensorimotor state (Engel & Fries, 2010).

In the past two decades, gamma oscillations have attracted considerable attention because of their putative importance for establishing functional relations among neuronal assemblies. Because of their high speed and occurrence throughout the brain, gamma oscillations have been proposed as a solution to the binding problem (Engel & Singer, 2001). A related function was proposed by Fries, who extended the notion of binding to the "communication through coherence" hypothesis, in which he stated that gamma activity can support synchronous activity and thereby communication between brain regions, which is characterized by the phase lag between the regions (Fries, 2005). This hypothesis was supported by a study in which several neuronal groups across visual areas engaged in gamma-band synchronization. The interaction strength between the groups was dependent on their phase relations (Womelsdorf *et al.*, 2007).

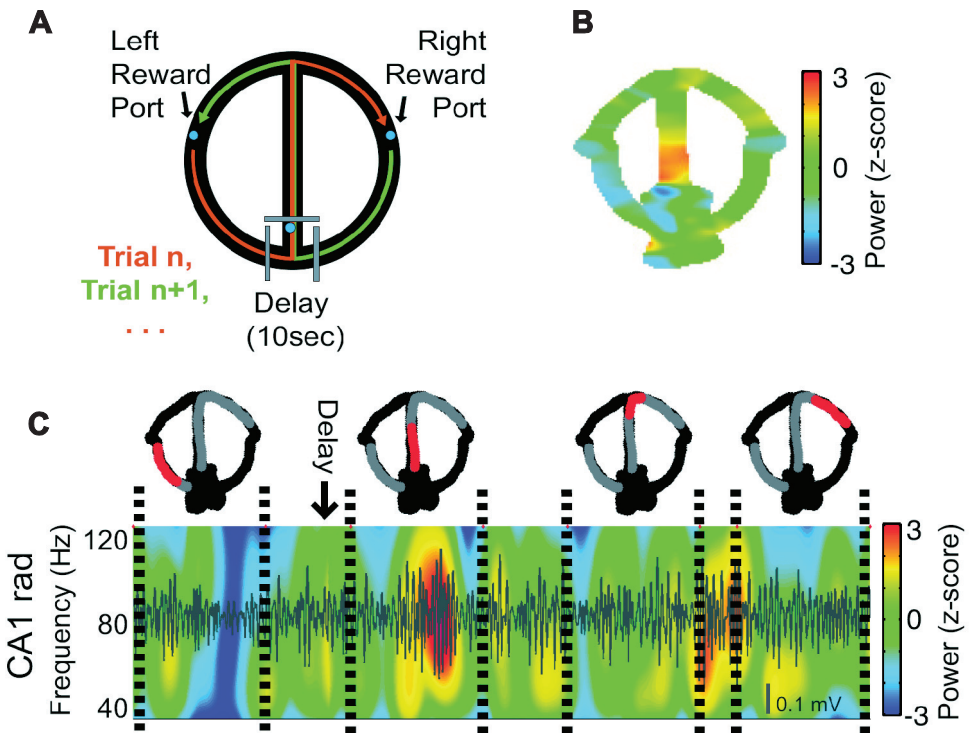

Figure 1.4. **Gamma power increases during spatial alternation task**.
A) Schematic representation of maze used for the spatial alternation task. Rats were rewarded when the chosen direction in upper crossing was alternated. B) Average gamma power in CA1 recorded during different phases of the alternation task. Note the increase in the center arm, where the animal has to remember which way to go is present C) Typical time-frequency plot of local field potential measured in hippocampus CA1 during alternation task. The gamma-band power increased when the animal was in the center arm. Adapted from (Montgomery & Buzsaki, 2007).

Many studies have established the importance of gamma oscillations in memory. In rats, hippocampal gamma oscillations increase during a spatial memory task (Fig. 1.4, Montgomery & Buzsaki, 2007). In macaque monkeys, increased gamma band synchronization between hippocampal neurons predicted enhanced memory performance (Jutras *et al.*, 2009). Gamma activity has also been associated with the formation of declarative memories in humans. Depth-EEG study in epilepsy patients showed that hippocampal gamma power increased during the successful encoding of new memories (Sederberg *et al.*, 2007), and that this increase correlates with memory load (van Vugt *et al.*, 2010). One way to think of gamma oscillations as having a role in memory formation, is that the phase of the oscillation will regulate the flow of action potentials with high temporal resolution, thereby supporting the formation of LTP (long-term potentiation), one of the mechanisms underlying memory (Dan & Poo, 2004; Axmacher *et al.*, 2006). In a study by Singer and colleagues, the effect of gamma oscillations on LTP in the

visual cortex was investigated (Wespatat *et al.*, 2004). They found that the exact timing of the excitatory post synaptic potentials (EPSPs) with respect to the gamma phase determined whether LTP or LTD (long-term depression) was induced, which indicates that there is an important role for gamma oscillations in synaptic modulation by LTP and LTD.

In summary, brain oscillations have been implicated with a variety of cognitive functions. The studies mentioned above have established phenotypic correlations between oscillations and cognition. This raises the question whether genes that influence brain oscillations also underlie cognitive traits.

1.4.2 Common genetic sources for oscillations and cognition or disease

Neuronal oscillations have successfully been used as endophenotypes for alcoholism (Rangaswamy & Porjesz, 2008). Resting-state beta power is increased in the EEG of alcoholics (frontal region, (Rangaswamy *et al.*, 2002), and a linkage study associated beta frequency with a set of genes coding for GABAA receptors (Porjesz *et al.*, 2002). Subsequently, a candidate gene association study revealed that polymorphisms in the GABRA2 gene predicted the risk for alcoholism (Edenberg *et al.*, 2004), a finding that was replicated several times (Covault *et al.*, 2004; Soyka *et al.*, 2008). Other linkage studies have found common genetic causes for alcoholism and alpha power (Winterer *et al.*, 2003; Ehlers *et al.*, 2010). The P300 EEG response amplitude is reduced in alcoholics, and a linkage study for the P300 response in the theta band identified the gene CHRM2, that codes for the muscarinic acetylcholine receptor M2 (Jones *et al.*, 2004). This gene was later associated with alcohol dependence (Wang *et al.*, 2004), and IQ (Gosso *et al.*, 2006; Dick *et al.*, 2007). Thus, by studying the genetics of brain oscillations, several genes have been identified that also play a role in alcohol dependence.

Neuronal oscillations have also been proposed as endophenotypes for schizophrenia. Several cognitive functions are impaired in schizophrenics, which may be mediated by altered oscillations (Uhlhaas & Singer, 2010). During a working memory task, gamma oscillations increase in healthy subjects, but in schizophrenics this increase is absent (Basar-Eroglu *et al.*, 2007). GABAergic signaling is essential for gamma oscillations (Whittington *et al.*, 1995; Bartos *et al.*, 2007; Sohal *et al.*, 2009). In mice, alterations of the gene GAD1, the major determinant for GABA levels, disrupts synchronization of hippocampal gamma oscillations (Fuchs *et al.*, 2001). The levels of mRNA for GAD67, encoded by the gene GAD1, are reduced in schizophrenics (Hashimoto *et al.*, 2003), and polymorphisms of GAD1 have been associated with schizophrenia (Straub *et al.*, 2007). Thus, gamma oscillations may mediate the effect of GAD1, or more generally of altered GABAergic signaling, in schizophrenics (Gonzalez-Burgos *et al.*, 2010).

Another gene associated with schizophrenia is NRG1, and its receptor ErbB4 (Stefansson *et al.*, 2002; Mei & Xiong, 2008). An *in vitro* study has shown that the amplitude of kainate-induced oscillations in the hippocampus was reduced

in the ErbB4 knockout-mice (Fisahn *et al.*, 2008). Thus, gamma oscillations may be an endophenotype relating the gene ErbB4 and schizophrenia, since gamma amplitude is reduced in schizophrenics (Buonanno, 2010).

As opposed to alpha amplitude, no genome wide linkage studies have been performed for gamma amplitude in humans, which is surprising since gamma oscillations are strongly related to several cognitive functions and psychiatric diseases. There is only one candidate-gene association study for gamma oscillations in humans, which reported a relation between auditory gamma responses amplitude and genes coding for dopamine receptor D4 (DRD4), and dopamine transporter (DAT), but not with COMT (Demiralp *et al.*, 2007). Thus, although gamma oscillations are a potential source of endophenotypes, because of their strong implication with several cognitive functions and psychiatric diseases, little is known about the genetics of gamma oscillations in humans.

Naturally, there are fewer ethical limitations for studying the genetics of brain activity in mice than in humans. Activity from specific brain regions and brain cells in mice can be measured and pharmacologically manipulated *in vivo* and *in vitro*. Also, genetically engineered mice facilitate the search for genes underlying, e.g., neurophysiological traits. Gamma oscillations can be induced in isolated brain tissue from different brain regions like the visual cortex (Anver *et al.*, 2010), hippocampus (Whittington *et al.*, 1997) and thalamo-cortical slices (Llinas *et al.*, 2007). This has led to the identification of several genes influencing gamma oscillations (Fuchs *et al.*, 2001; Hormuzdi *et al.*, 2001; Llinas *et al.*, 2007; Fisahn *et al.*, 2008). However, also for these kinds of recordings there has never been an unbiased genome wide scan for genes influencing gamma oscillations. Moreover, the oscillations were merely characterized with frequency, amplitude and/or correlation measures even though their rich spatiotemporal dynamics possibly requires a broader range of traits to fully capture their dependence on different genes or involvement in cognitive processing.

For the research in this thesis, we explored both classical and more recently developed algorithms to characterize carbachol-induced hippocampal oscillations *in vitro* in genetically engineered mice to perform genome-wide scans for genes that influence these oscillations.

1.5 Hippocampal oscillations *in vitro*

In vitro gamma oscillations in rat hippocampus were induced for the first time in the nineties by applying a condition train of stimuli at 100 Hz to afferent fibres for 1 second, at half the voltage needed to evoke an action potential (Miles & Poncer, 1993; Whittington *et al.*, 1995; Whittington *et al.*, 1997). These oscillations were found to be dependent on metabotropic glutamate receptors (mGluRs), and this led to the idea to induce oscillations pharmacologically by glutamate (Whittington *et al.*, 1995). Glutamate is an agonist that activates glutamate receptors, thereby increasing network activity. In hippocampal slices, gamma oscillations can also be induced by activating other types of receptors using agonists such as DHPG

(Pálhalmi *et al.*, 2004), kainate (Hormuzdi *et al.*, 2001; Fisahn *et al.*, 2004) or carbachol, which is a cholinergic agonist that activates acetylcholine receptors (Fisahn *et al.*, 1998). Acetylcholine is a major neurotransmitter involved in learning and memory (Hasselmo, 2006), therefore we focused on carbachol-induced oscillation in the research presented in this thesis.

1.5.1 Carbachol-induced hippocampal oscillations

Muscarinic acetylcholine receptors (mAChRs, M_1– M_5) are metabotropic receptors. They are named after the natural product muscarine, which was first found in the mushroom Amanita muscaria. Muscarine mimics the effect of the neurotransmitter acetylcholine, a cholinergic agonist for all 5 types of mAChR. The hippocampus receives cholinergic input from the brain stem, and muscarinic blockade reduces the amplitude of hippocampal gamma *in vivo* (Hentschke *et al.*, 2007). Carbachol is another cholinergic agonist of all mAChRs, that induces fast network oscillations when applied to hippocampal slices *in vitro* (Fisahn *et al.*, 1998).

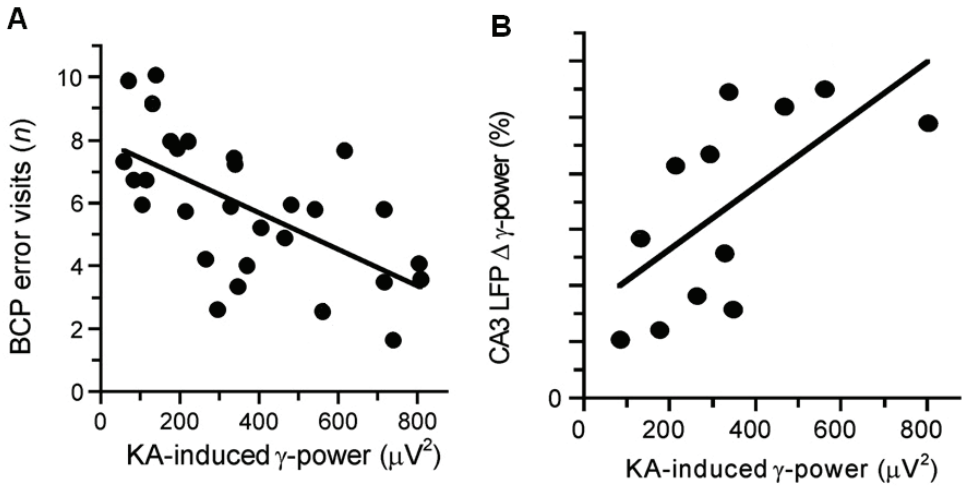

Figure 1.5. **Gamma power *in vitro* correlates with gamma power *in vivo* and performance in a spatial memory task.**
C57bl/6J mice were subjected to a memory task and hippocampal local field potential (LFP) measurements *in vivo* and *in vitro.* (A) Kainite (KA) -induced gamma power from hippocampal slices correlates negatively with the number of errors made in a spatial memory task in the Barnes circular platform (BCP). (B) *In vivo* CA3 local field potential (LFP) gamma power was measured during inactive and active states of the mice. The activity-induced increase in CA3 LFP power as percentage of total power in the inactive state increases with *in vitro* gamma power. Adapted from (Lu *et al.*, 2010a).

In vivo, the hippocampus contains at least two gamma oscillation generators: one in CA3 and one in the dentate gyrus (Csicsvari *et al.*, 2003). Also, *in vivo* hippocampal gamma oscillations are often nested in theta waves, which are

produced by multiple generators and have been shown to have multiple behavioral correlates (Montgomery *et al*., 2009). In the reduced *in vitro* preparation, however, only the CA3 gamma generator is active, and theta activity is not always present. The *in vitro* oscillations share many characteristics with the *in vivo* CA3 gamma generator, such as amplitude and phase distributions over hippocampal subregions (Csicsvari *et al.*, 2003; Mann *et al.*, 2005b). Whether the amplitude of carbachol-induced oscillations *in vivo* correlates positively with amplitude of *in vivo* hippocampal oscillations is not known, but recently this has been shown to be the case for kainite-induced oscillations (Fig. 1.5) (Lu *et al.*, 2010a). Thus, *in vitro* hippocampal oscillations are regarded as a good model system for investigating mechanisms of the CA3 gamma oscillator, and it has been used extensively in the past decade.

Several studies have investigated cellular mechanisms of *in vitro* hippocampal gamma oscillations. Current-source density analysis revealed sink/source pairs in the CA3-stratum pyramidale and CA3-stratum radiatum. Simultaneous recording of local field potential and voltage sensitive dye imaging revealed that the active current sinks and sources are generated in the stratum pyramidale, generating mainly passive return currents in the stratum radiatum (Mann *et al.*, 2005b). These findings suggested an active participation of the pyramidal cells, which was confirmed by intracellular recordings. Both pyramidal cell spike activity and membrane potential exhibit strong phase-locking with the field potential oscillations. Interneurons also fire phase-locked with the field potentials, albeit with a slightly longer phase lag than the pyramidal cells. These observations supported a model in which pyramidal neurons excite interneurons, and interneurons inhibit pyramidal cells perisomatically in a recurrent feedback loop. Computer models of neuronal networks have shown that such a recurrent feedback loop is sufficient to create gamma oscillations (Mann *et al.*, 2005a). To further validate this model, excitatory and inhibitory postsynaptic currents (EPSPs and IPSPs) were measured in pyramidal cells and different types of interneurons (Oren *et al.*, 2006). Input to pyramidal cells appeared to be mostly inhibitory, whereas input to interneurons was mostly excitatory, which supported the proposed model.

Different interneurons differ in their firing rates and phase coupling to the local field potential (Hájos *et al.*, 2004; Oren *et al.*, 2006; Klausberger & Somogyi, 2008; Gonzalez-Burgos *et al.*, 2010). Parvalbumin-positive interneurons target perisomatically and are the most abundant interneuron class comprising about 20% of the total population. These neurons have been shown repeatedly to play an important role in the generation of gamma oscillations in rodents *in vitro* (Vreugdenhil *et al.*, 2003) and *in vivo* (Fuchs *et al.*, 2007; Lodge *et al.*, 2009; Wulff *et al.*, 2009), and in humans (Sohal *et al.*, 2009).

In order to find genes that influence hippocampal network activity, pharmacological manipulations and genetically engineered mice have been employed. It appeared that carbachol-induced hippocampal oscillations are blocked by the mAChRs antagonist atropine, but also by the M_1-specific antagonist

pirenzepine (Fisahn *et al.*, 1998), which suggests that the activity depends specifically on the activation of the M_1 receptor. This idea was supported by the finding that muscarine failed to induce hippocampal oscillations in M_1-deficient mice (Fisahn *et al.*, 2002). In this same study it was shown that in mice lacking one of the other four mAChRs, muscarine did induce oscillations. By applying several antagonists (to block specific receptor types), it has been established that carbachol-induced oscillations do not depend on metabotropic glutamate and NMDA receptors, but do depend on $GABA_A$ and AMPA receptors, (Fisahn *et al.*, 1998; Pálhalmi *et al.*, 2004). Oscillations induced by kainate or glutamate also depend on $GABA_A$ receptors. Several studies have analyzed which specific $GABA_A$ receptor subunits are involved, and reported that the Gabra5 knockout-mice have larger amplitudes (Glykys *et al.*, 2008), and Gabrd and Gabrb3 knockouts have higher frequency (Hentschke *et al.*, 2009; Mann & Mody, 2010).

Taken together, several genes have been shown to influence frequency and/or amplitude of hippocampal gamma oscillations. It remains largely unknown, however, whether other properties of these oscillations are heritable, and which other genes play a role in regulating network activity in the hippocampus. In the research presented in this thesis, we have performed an unbiased genome wide search by means of QTL mapping, to search for novel candidate genes that influence hippocampal oscillations.

1.6 Relating genotype with quantitative traits using QTL mapping

A quantitative trait can take values from a continuous range, as opposed to a discrete trait such as gender. Quantitative traits are often influenced by many environmental and genetic factors, which explains its continuous distribution. A quantitative trait locus (QTL) is a stretch of DNA, often containing many genes, that influences the quantitative trait. In animal studies, crosses of inbred strains, and other breeding schemes have been used to identify QTLs (Flint *et al.*, 2005). Examples of crosses used for QTL mapping are F2 (obtained by intercrossing an F1, which is the crossing of two inbred strains), congenic (yields mice with one genomic region from one inbred strain and the rest of the genome from another inbred strain), and recombinant inbred strains. Recombinant inbred strains are generated by inbreeding the progeny of two inbred strains, to create a panel of inbred strains, each with a unique combination of the genomes of the parental strains. The two inbred stains that are used to create recombinant inbred strains are often well studied strains that are known to differ in the trait of interest. Examples are the AXB panel (A/J crossed with C57BL/6J, known to differ in neural proliferation (Poon *et al.*, 2010)), the BXH panel (C57BL/6J crossed with C3H, know to differ in their resistance to hyperthyroidism (McLachlan *et al.*, 2008)) and the BXD panel (C57BL/6J crossed with DBA/2J), which is the largest and most frequently used inbred strain panel.

1.6.1 Using BXD recombinant inbred strains to identify QTLs

The panel of BXD strains was recently expanded to 80 strains (Peirce *et al.*, 2004) and it has been used to identify QTLs for a variety of traits such as the size of different brain regions, body weight, alcohol-sensitivity and several other behavioral traits. In particular, alcohol-sensitive behavioral traits have been studied widely in the BXD panel, because the two parental strains differ markedly in a variety of alcohol responses (Crabbe, 2002). For sensitivity to alcohol withdrawal, a few QTLs have been identified using BXD strains (Buck *et al.*, 1997), which were narrowed down to a small interval using congenic strains (Fehr *et al.*, 2002), and further to the identification of the gene MPDZ, with the use of gene expression data (Shirley *et al.*, 2004).

The C57BL/6J and DBA/2J strains also differ in many neurophysiologic hippocampal traits and hippocampus-related behavioral traits. For example, C57BL/6J outperforms DBA/2J in spatial memory tasks (Crusio *et al.*, 1987; Ammassari-Teule *et al.*, 1995; Passino *et al.*, 2002), which has been associated with their differences in synaptic plasticity (Nguyen *et al.*, 2000), and hippocampal mossy fiber projections (Schwegler & Crusio, 1995; Schwegler *et al.*, 1996; Middei *et al.*, 2007). The BXD strains, therefore, form an excellent resource to identify the segregating genetic variants that affect hippocampal network activity.

The identification of QTLs is often considered as a first step to find the gene(s) in the QTL that cause the phenotypic variance. However, the identification of genes is a daunting task: a meta-analysis has shown that for the 2050 QTLs identified until 2005, only around 20 candidate genes were detected (Flint *et al.*, 2005). The same article proposes strategies to increase the likelihood of identifying the relevant genes in QTLs, such as the use of larger inbred panels, outbred stocks or system genetics approaches by using, for example, gene expression data. For BXD strains, expression levels in many different tissues have been measured (Overall *et al.*, 2009). For our research we used the BXD panel to identify QTLs for hippocampal network activity. The QTLS were narrowed down to a few candidate genes by using hippocampal gene expression measured in the BXD strains.

1.6.2 Genetic correlation between hippocampal activity and behavioral traits

Several studies have shown that hippocampal gamma oscillations increase in amplitude during memory tasks (Sederberg *et al.*, 2007; van Vugt *et al.*, 2010). It is not known, however, whether the predisposition for producing high-amplitude gamma oscillations is also associated with enhanced cognitive capacity, such as better performance in a memory task. In psychiatric studies, reduced but also increased EEG gamma amplitude have been observed in patients. In schizophrenia, reduced gamma amplitude is associated with negative symptoms (working memory impairment) and increased gamma amplitude with positive symptoms

(hallucinations) (Uhlhaas & Singer, 2010). In Alzheimer patients, gamma amplitude is reduced, and ADHD and epilepsy patients have high EEG gamma amplitude (Herrmann & Demiralp, 2005). From these EEG studies it is clear that high gamma amplitude is not always beneficial.

Measurement of gamma band activity during rest in children showed that gamma amplitude increases during early child development (Takano & Ogawa, 1998; Uhlhaas *et al.*, 2008) and is positively correlated with cognitive and language skills in children at the age of 1-3 (Benasich *et al.*, 2008). In adults, to our knowledge only one study has tried to link gamma amplitude with cognitive ability, which showed that emotional intelligence is positively correlated with gamma amplitude (Jausovec & Jausovec, 2005).

In mice, it was shown only recently that *in vitro* kainite-induced gamma amplitude correlates with *in vivo* gamma amplitude, and with performance in a memory task (Lu *et al.*, 2010a). The same study showed that spontaneous *in vitro* gamma activity correlates with *in vivo* gamma activity during rest. Thus, it is plausible that the traits we measured from hippocampal oscillations and spontaneous activity in mice are correlated with the equivalent *in vivo* traits and the behaviors they support. However, Lu *et al.* used genetically identical mice, which implies that the observed variation in amplitude can only be caused by environmental factors, and in this case we call the relation between amplitude and memory performance "environmental correlation". This raises the question whether these traits also have a genetic correlation, i.e., are there genes that influence both gamma amplitude and memory performance or other cognitive tasks. We are not aware of any study addressing this question. In this thesis, we measured amplitude and other traits of hippocampal network activity in a panel of BXD strains, which allowed us to compute genetic correlations between the hippocampal traits and behavioral traits previously measured in other BXD studies, among which performance in a memory task.

1.7 Quantitative analysis of hippocampal network activity

Local field potentials (LFP) reflect a superposition of extracellular activity from hundreds to thousands of neurons surrounding the recording electrode. We measured LFP with an 8-by-8 multi electrode grid with 200 µm inter-electrode distance, which covered a transverse slice of the mouse hippocampus. The signal was recorded at 1000 Hz and down-sampled to 200 Hz for off-line analysis.

1.7.1 Classical traits of neuronal network activity

Hippocampal LFP activity is traditionally characterized using Fourier-based spectral analysis. The Fourier transform decomposes a signal in sinusoidal waves with different amplitudes and frequencies. This is often visualized in an amplitude spectrum, a graph of frequencies versus the corresponding amplitudes. If a signal is dominated by oscillations, this shows up in the spectrum as an amplitude peak in

the frequency band at which the oscillations occurred. We recorded oscillatory and non-oscillatory signals. Non-oscillatory signals exhibited *1/f*-like spectra, and we characterized them by the integrated amplitudes for several frequency bands. Oscillatory signals displayed spectra with a clear peak on top of the *1/f* curve. From these spectra we computed integrated amplitudes for several frequency bands, as well as the peak frequency and the peak amplitude (the location and the height of the peak in the spectrum, respectively).

The hippocampus forms a strongly interconnected network in which different subregions are believed to communicate and coordinate information processing partially on the basis of neuronal oscillations. This exchange of information can be detected and quantified using a variety of cross-correlation techniques. The cross-correlation function measures the correlation between two signals as a function of a shift in time of one of the signals. If two signals are correlated with a certain time-delay, this shows up as a peak at that time lag in the cross-correlogram. If such a time delay is not present, the cross correlation is highest at zero time shift, which is the correlation. The non-oscillatory signals had the highest correlation at zero time shift, therefore we quantified the interactions between these signals with correlation.

The phase-locking factor (PLF) is a well established measure for quantifying the interaction between two oscillating signals that can be out of phase and possibly have independent amplitude fluctuations (Tass *et al.*, 1998; Lachaux *et al.*, 1999). For example, two sinusoidal waves with independent amplitude fluctuations may have a low cross correlation, but a high PLF. To compute the PLF of two oscillatory signals, the instantaneous phase (i.e., the phase at each time point of sampling) is estimated for each signal with the Hilbert transform or wavelet analysis (Le Van Quyen *et al.*, 2001). If *p1(t)* is the phase of the first signal, and *p2(t)* the phase of the second, the instantaneous phase lag between the two signals is *p2(t)-p1(t)*. The distribution of these phase lags is used to compute the PLF and the phase lag at which the two signals are locked. The interactions of the oscillatory signals were characterized by the PLFs and corresponding phase lags.

1.7.2 Non-classical traits

In addition to the classical methods we also used analysis techniques of which some, to the best of our knowledge, never have been applied to hippocampal LFPs.

The phase-locking factor technique provides besides the PLF, also the phase lag at which the two signals are locked. We computed phase lags between the hippocampal subregions CA3 stratum radiatum/lacunosum moleculare (CA3SR) and CA3 stratum oriens (CA3SO), and between subregions CA3SR and CA1SR (see Chapter 4, Fig. 1C). Hippocampal gamma oscillations are assumed to be generated by a feedback loop between CA3S and CA3SR (Mann *et al.*, 2005b), the phase relation between these regions is a characteristic of this loop. After being generated in CA3, the oscillations travel to CA1. The phase lag between CA3SR and CA1SR reflects the speed with which the oscillations travel.

The phase-locking technique can also be used to compute the locking between two signals that oscillate at different frequencies *n* and *m* (Tass *et al.*, 1998). In this case, the phase lags are computed by taking *n*p2(t)-m*p1(t)*. This method can also be applied to a single signal, by applying a pass-band frequency filter around each of the two frequencies *n* and *m*, thereby creating two signals for which the so called cross-frequency phase-locking factor and phase lag are computed (Palva *et al.*, 2005).

If the Fourier amplitude spectrum of a signal contains peaks at two frequencies, and the higher frequency is twice the low peak frequency, this can mean two things. First, the signal may contain two independent oscillations that happen to be a multiple of each other (Nikulin & Brismar, 2006). Second, if the oscillation at the lower frequency has a shape that diverges from a sinusoidal wave, the second peak at twice the frequency might be the so-called harmonics of the oscillation, which is the way the Fourier transform compensates for the quasi-sinusoidal shape. In the latter case, the cross-frequency PLF and phase lag characterize the quasi-sinusoidal shape of the oscillations. The local field potentials we measured (see Materials and Methods, Chapter 4) contain oscillations with a main frequency at around 20 Hz, and the amplitude spectrum often contains a second peak at twice the main frequency (see Chapter 4, Fig. 1D). This second peak in the amplitude spectrum is the harmonics of the main frequency, because the oscillations at the mean frequency have a quasi-sinusoidal shape (see Chapter 4, Fig. 1E), and the amplitudes at the two frequencies are strongly correlated over time. Moreover, an independent generator of oscillations with a frequency exactly twice the main frequency has never been observed to our knowledge. Thus, we computed the cross-frequency PLF and phase lag in order to characterize the quasi-sinusoidal shape of the oscillations, which may reflect physiological properties of the mechanisms that underlie the oscillations.

Detrended fluctuation analysis (DFA) has been used in human EEG studies to characterize amplitude dynamics (Linkenkaer-Hansen *et al.*, 2001). For this analysis, the signal is filtered around the peak frequency, and the amplitude envelope of the signal is extracted (that is, an estimate of the amplitude at each sample). DFA estimates the decay of auto-correlations of the amplitude envelop. Since the presence of auto-correlations can be viewed as a memory in the signal, the DFA has been proposed to index neuronal processing relevant for memory (Montez *et al.*, 2009).

Oscillation burst life time analysis can also be performed on the amplitude envelop. An oscillation burst begins when the amplitude envelope passes above a certain threshold, and ends when it passes below the threshold. The oscillation bursts were characterized by the 95-percent percentile of the cumulative distribution of the life-times.

LFPs are the sum of stochastic and deterministic factors. Langevin equations allow to decompose signals into the deterministic and stochastic components, and provides models with several parameters for these components.

For the cross-frequency traits, the DFA exponent and the lifetime traits, we used the electrode that measured the highest peak amplitude. For the Langevin parameters we also used only one electrode, the selection of this electrode is described in Chapter 3.

1.8 Aims of this thesis

Neuronal oscillations in the gamma frequency band are implicated in several cognitive functions and psychiatric diseases. Task related gamma amplitude has been found to be different between individuals and this variation is to a large extent due to genetic factors, from which only few have been identified. In mice, the molecular machinery involved in the genesis of gamma oscillations can be studied using slice preparations, pharmacological manipulations, and genetically engineered mice. This has led to the identification of several genes influencing hippocampal gamma oscillations. However, an unbiased genome wide search for this type of brain activity has never been performed. Moreover, most of the genes identified were related to the classical traits frequency, amplitude and coherence of hippocampal oscillations, but hippocampal network activity can be characterized in many ways, and different properties may be related to different genes and different parts of the cognitive spectrum. As indicated at the beginning of this chapter, the aims of this thesis to 1) thoroughly characterize hippocampal network activity with classical and non-classical quantitative traits 2) estimate the heritability of these traits 3) perform a genome wide search for genes that influence the hippocampal traits and 4) investigate with which behavioral or cognitive traits the hippocampal traits may be related. The following steps have been taken to reach these goals:

1) Long-range temporal autocorrelations of alpha amplitude fluctuations in humans have been characterized by the DFA exponent, which appeared to be highly heritable and different for Alzheimer patients compared to healthy subjects. It was unknown if *in vitro* oscillations also exhibit significant long-range temporal correlations, and whether these correlations can be modulated by pharmacological manipulations. In Chapter 2 we compute DFA exponents of hippocampal oscillations *in vitro*, induced with different concentrations of carbachol, to address these questions.

2) Hippocampal local field potentials are the sum of stochastic and deterministic factors. Langevin equations allow to decompose signals into deterministic and stochastic components, and provide models with several parameters for these components. In Chapter 3, we estimate these parameters for non-oscillatory hippocampal activity in order to 1) investigate whether these parameters differ between hippocampal subregions and 2) estimate the heritability of these parameters with the use of measurements in inbred strains.

3) In Chapter 4, we estimate the heritability and genetic correlations of classical traits of oscillatory and non-oscillatory hippocampal activity: amplitude in several frequency bands and hippocampal subregions, and inter-regional correlations. Measurements in eight inbred strains allowed for estimation of heritability. Genetic correlations were computed in order to see to what extent these different properties were influenced by the same or by different genes. Importantly, finding a significant heritability is a prerequisite for a subsequent genome wide search to be successful.

4) In Chapter 5, we perform a genome wide search for the classical traits studied in Chapter 4. This was done by measuring hippocampal network activity in a panel of 29 BXD recombinant inbred strains. A system genetic approach using QTL mapping and gene-expression was applied to search for novel gene candidates. In addition, a large database of phenotypes measured in BXD strains was used to compute genetic correlations between the hippocampal activity traits and a wide range of behavioral phenotypes.

5) Chapter 6 contains the heritability estimates and genome wide searches for the non-classical traits, the genetic correlations between the complete set of classical and non-classical traits, and the genetic correlations between traits from this complete set and the database of behavioral phenotypes.